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Legume Lectins

I. IMMUNOLOGICAL CROSS-REACTIONS BETWEEN THE ENZYMIC LECTIN FROM MUNG BEANS AND OTHER WELL CHARACTERIZED LEGUME LECTINS[1]

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ABSTRACT

A number of well characterized legume lectins including the enzymic lectin from *Vigna radiata* were examined for immunological relatedness. The immunological cross-reactions observed indicate that most of the legume lectins, including *Vigna* lectin, are evolutionarily closely related proteins. The possibility that these proteins are homologs with enzymic functions is discussed.

Recently we discovered a protein in mung bean seeds which possessed both lectin and enzymic properties (5). This finding led us to ask if this enzymic lectin was related to any of the other legume lectins which have been described. The possible significance of a relationship, with respect to understanding the functional role(s) of plant lectins, is obvious.

Lectins appear to be widely distributed among leguminous plants (13) and most of what we now know about these proteins is the result of studies with lectins isolated from species of this plant family. Collectively, the legume lectins display many similar properties. For example, most of those thus far studied are metal ion-binding, tetrameric glycoproteins possessing identical or nearly identical subunits (8, 10). Based upon their common source and upon their possessing similar physical, chemical, and biological properties, many of the legume lectins might be homologs (4).

Immunochemistry provides a powerful method for determining relationships among proteins. Different proteins can be functionally similar and yet antigenetically totally unrelated. However, when proteins are found to be antigenetically similar there is a strong probability of both an evolutionary and a functional relationship. We have begun experiments designed to assess the immunological relatedness both among legume lectins and between these proteins and the enzymic lectin from mung beans. Initial studies in this laboratory (6) indicated that many of the plant lectins which have been previously studied are immunologically related and, moreover, that there may be a unique portion of these proteins that is evolutionarily highly conserved.

In this report we provide evidence that the enzymic lectin from mung beans is immunologically closely related to several other legume lectins and that these lectins are members of a group of related proteins that encompass the majority of the legume lectins which have been studied.

MATERIALS AND METHODS

Lectins. The lectins used in this study were derived from the plant species listed in Table I. The sources of the lectins and their hapten sugar specificities are indicated. All of these species with the exception of *Ricinus communis* (castor bean) are from the family Leguminosae. This list represents the majority of the legume lectins which have been well characterized.

Antisera. High titer rabbit antisera were raised against the lectins from *Vigna radiata*, *Sophora japonica*, *Bandeiraea simplicifolia*, *Bauhinia purpurea alba*, *Glycine max*, and *Phaseolus lunatus* by methods described previously (7). The lectin preparations used as antigens were homogeneous or nearly homogeneous as judged by SDS gel electrophoresis. We have previously shown (6) that antisera derived by these methods react specifically with lectins, but not with other seed proteins.

Double Diffusion. Agar double diffusion experiments were done using a 10-μl sample size according to the method of Ouchterlony (8). Sugars (galactose, fucose, and glucose) were incorporated into the agar (at 50 mM concentrations) during the preparation of slides to prevent any interaction between lectins and carbohydrate containing serum components.

Neutralization of Hemagglutinin Activity. The ability of antisera to inhibit lectin-induced erythrocyte agglutination was estimated by comparing the minimum concentration of lectin required for agglutination of trypsinized rabbit red blood cells (9) in the presence of normal sera to that in antisera. Serial dilutions of lectin samples (25 μl) were made in normal sera or antisera and allowed to incubate for 10 min at room temperature. An equal volume (25 μl) of trypsinized rabbit red blood cells (2% suspension phosphate-buffered saline) was then added and after 60 min at room temperature the titer was recorded as the reciprocal of the highest dilution showing agglutination when examined with the aid of a binocular microscope.

RESULTS

All of the lectins which were available to us were screened by Ouchterlony double diffusion against normal sera and antisera prepared against pure lectins from six legume species. The tests were done on agar plates containing sugars to prevent any spurious lectin-glycoprotein interactions. Figure 1 shows the results of a typical set of tests using *Bauhinia* antisera. In no cases under these conditions, were precipitin lines obtained with preimmune sera. In all cases where a cross-reaction was observed, a single "spur" was present indicating that the cross-reacting lectin was immunologically related but not identical to the homologous lectin.

The results of all of these screenings are summarized in Table II. Note that 11 of the 13 galactose-specific lectins tested cross-reacted with one or more of the six antisera. There are sufficient overlapping cross-reactions to say that at least 12 of these 13 proteins are related. Pure *Vigna* lectin gave precipitin lines with three of the five heterologous antisera used. Antisera raised against *Vigna* lectin recognized six galactose-specific legume lectins and the castor bean lectin (Fig. 2).

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Table I

Lectin	Source*	Sugar Specificity
1. Arachis hypogaea (peanut)	1	Galactose
2. Bandeiraea simplicifolia	1	Galactose
3. Bauhinia purpurea alba	2	N-acetylgalactosamine
4. Dolichos biflorus	1	N-acetylgalactosamine
5. Glycine max (Soy bean)	1	N-acetylgalactosamine
6. Lotus tetragonolobus	2	Fucose
7. Phaseolus lunatus (Lima bean)	2	N-acetylgalactosamine
8. Phaseolus vulgaris (PHA-E; Kidney bean)	1	Galactose
9. Ricinus communis (Castor bean)	1	Galactose
10. Sophora japonica (Pagoda tree)	1	N-acetylgalactosamine
11. Ulex europeus (Gorse)	1	Fucose
12. Vigna radiata (Mung bean)	3	Galactose
13. Wisteria floribunda	2	N-acetylgalactosamine
14. Conavalia ensiformis (Jack bean)	1	Glucose/mannose
15. Lens culinaris (Lentil bean)	1	Glucose/mannose
16. Pisum sativum (Garden pea)	1	Glucose/mannose

* Sources were: 1, Vector Labs, Burlingame, CA.;
2, E-Y Labs, San Mateo, CA;
3, Purified by methods described previously (1).

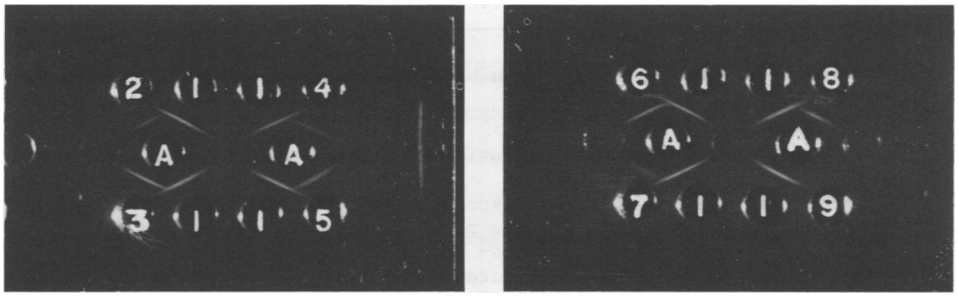

FIG. 1. Typical immunological cross-reactions. A: *Bauhinia* antiserum, 10 μl; 1: *Bauhinia* lectin, 10 μg; 2: *P. vulgaris* (E), 10 μg; 3: *Sophora*, 10 μg; 4: *Wisteria*, 200 μg; 5: *Ulex*, 200 μg; 6: *Bandeiraea*, 10 μg; 7: *Lotus*, 10 μg; 8: *Dolichos*, 10 μg; 9: *Vigna*, 10 μg. Photograph taken after 4 h at 37 C.

As a further test of both the strength and specificity of the cross-reactions, we examined the ability of the antisera to inhibit lectin-induced hemagglutination (Table III). The hemagglutinating activity of every lectin which cross-reacts with an antiserum is also specifically inhibited by that serum (compare Tables II and III). The inhibitory potency of each serum was for the most part closely related to the relative strength of the cross reactions seen by double diffusion. That is, each antiserum most potently inhibited the hemagglutinin activity of those lectins with which it most strongly cross-reacted. In no case where we could make the test did we observe a cross-reaction between serum and lectin without also seeing inhibition of lectin activity by that serum. We did, however, observe significant inhibition with antisera in three cases without also seeing a precipitin line following double diffusion.

DISCUSSION

All of the legume lectins that have been characterized to date can be classified into two main groups with respect to their carbohydrate-binding specificities: (*a*) those specific for galactose (and its derivatives such as fucose and GalNAC); and (*b*) those specific for glucose or mannose (and their derivatives). The overwhelming majority of the legume lectins that have been studied fall into the galactose-specific group (Table I).

The results presented here indicate that there is extensive immunological homology among the lectins in the galactose group. Of 13 galactose-specific lectins tested, only peanut and soybean aggutinins failed to show a cross-reaction with at least one of the sera used (antisera against soybean lectin did, however, react with two other lectins). We believe that these results coupled with what is already known about these proteins (they are all lectins, they have similar chemical and physical properties, they are all glycoproteins, they are all from the same plant family, and they are all isolated from seeds) suggest that many of the galactose lectins from legumes are homologs. A similar conclusion has been reached (2–4, 10, 11) from comparative amino acid sequence analysis and, although these studies involved a very limited number of lectins, the similarities in N-terminal amino acid sequences of several galactose lectins was striking.

In addition to the immunological cross-reactions among most legume lectins, we also observed cross-reactivity between certain legume lectins and a lectin from a dicotyledonous plant (castor bean) outside the legume family. The galactose-specific castor bean lectin has many properties in common with the legume lectins and is also isolated from seeds. We believe that these results suggest that evolutionary and functional homologs of a unique lectin species occur widely (maybe even ubiquitously) in legumes and perhaps even in dicotyledonous plants in general. We have not observed any immunological relationship between the galactose lectins and lectins displaying other specificities but evidence consistent with an evolutionary relationship has been presented (3).

Our observation that the enzymic lectin from mung beans is closely related to many other galactose-specific legume lectins

Table II. Immunological cross-reactions between legume lectins

Lectin	Antisera: Bandeiraea Simplicifolia	Bauhinia purpurea alba	Glycine max	Phaseolus lunatus	Sophora japonica	Vigna radiata
1. Arachis hypogaea (peanut)	-	-	-	-	-	-
2. Bandeiraea simplicifolia	*	+	-	-	+	+
3. Bauhinia purpurea alba	+	*	-	+	+	+
4. Dolichos biflorus	-	+	+	+	+	+
5. Glycine max (Soybean)	-	-	*	-	-	-
6. Lotus tetragonolobus	+	+	-	-	+	+
7. Phaseolus lunatus (Lima bean)	+	-	+	*	-	-
8. Phaseolus vulgaris (PHA-E; Kidney bean)	+	+	-	-	-	-
9. Ricinus communis (Castor bean)	+	+	-	+	+	+
10. Sophora japonica (Pagoda tree)	+	+	-	-	*	+
11. Ulex europeus (Gorse)	+	+	-	-	+	+
12. Vigna radiata (Mung bean)	+	+	-	-	+	*
13. Wisteria floribunda	-	+	-	-	+	-
14. Conavalia ensiformis (Jack bean)	-	-	-	-	-	-
15. Lens culinaris (Lentil bean)	-	-	-	-	-	-
16. Pisum sativum (Garden pea)	-	-	-	-	-	-
Total positive crm	8	9	2	3	8	7

* = homologous reaction; + precipitin band observed;
- no precipitin band observed.

Table III. Inhibition of hemagglutination by antisera

Experiments were performed as described in "Methods." Numbers represent the ratio of the hemagglutinin titer in the presence of normal sera (control) to that in the presence of antisera. The number 1, then, indicates no inhibition by antisera, whereas large numbers indicate potent inhibition. Due to the qualitative aspects of this assay, little significance is attached to modest ratios, such as 2, 4, 8 although they could be real reflections of weak inhibition.

Lectin	Antisera: Bandeiraea Simplicifolia	Bauhinia purpurea alba	Glycine max	Phaseolus lunatus	Sophora japonica	Vigna radiata
1. Arachis hypogaea	4	1	4	1	4	1
2. Bandeiraea simplicifolia	*	64	1	1	4000	2000
3. Bauhinia purpurea alba	250	*	1	64	500	1000
4. Dolichos biflorus	4	64	500	128	500	128
5. Glycine max (Soybean)	2	4	*	500	64	1
6. Lotus tetragonolobus	-	-	-	-	-	-
7. Phaseolus lunatus	16	2	128	*	1000	1
8. Phaseolus vulgaris (E)	8	8	1	1	8	8
9. Ricinus communis	64	-	-	-	32	125
10. Sophora japonica	500	32	1	1	*	1000
11. Ulex europeus	-	-	-	-	-	-
12. Vigna radiata	32	2000	1	2	250	*
13. Wisteria floribunda	16	64	1	4	250	16
14. Conavalia ensiformis	-	-	-	-	-	-
15. Lens culinaris	1	1	1	1	4	1
16. Pisum sativum	1	1	1	1	4	1

* homologous antisera were totally (100%) inhibitory at the highest lectin concentrations used (1 mg/ml).
- Not done; Lotus and ulex lectins did not agglutinate rabbit RBCs under the assay conditions.

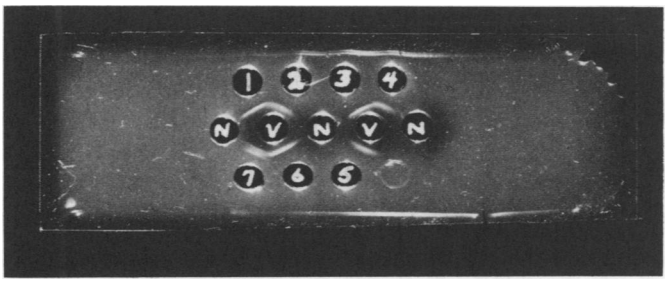

FIG. 2. Immunological cross-reactions between several lectins and antisera against *Vigna* lectin. N: normal rabbit serum, 10 μl; V: anti-*Vigna* serum 10 μl; 1: *Bandeiraea*, 10 μg; 2: *Bauhinia*, 10 μg; 3: *Dolichos*, 100 μg; 4: *Lotus*, 10 μg; 5: *Ulex*, 10 μg; 6: *Sophora*, 10 μg; 7: *Ricin*, 90 μg. Photograph taken after 6 h at 37 C.

suggests that all of these proteins may be homologous or very closely related enzymes. We are aware of the fact that none of the well characterized legume lectins has ever been shown to possess an enzymic activity comparable to that seen with mung bean lectin, although evidence that they might function in this manner has been reported (1). We have examined all of the commercially available legume lectins for simple glycolytic hydrolase activities and have found none. Thus, if these lectins are enzymes they are either very unstable (with respect to enzymic activity but not carbohydrate-binding activity) or they exist in the dry seed in an enzymically inactive form.

In a recent report Paus and Steen (12) describe and mannosidase from *Phaseolus vulgaris* which possesses lectin properties. Since the major lectin activity (PHA) from *P. vulgaris* is a galactose-type lectin, these results indicate that an individual plant species can possess lectin activities representative of both of the major specificity classes (galactose or glucose/mannose). This finding, coupled with that from mung beans, provides at least one example of a legume lectin from each specificity class which also possesses enzymic activity.

The evidence now available suggests that most of the well characterized legume lectins are members of a specific class of evolutionarily related legume proteins. Many of these lectins are likely to be homologs. In a following report we will provide evidence that this specific class of proteins is probably ubiquitous in the legume family. Since the enzymic lectin from *Vigna* is clearly a member of this protein class it is not unreasonable to assume that most if not all galactose-specific legume lectins have enzymic functions. We do not wish to imply that legume lectins might not have a function(s) which is nonenzymic in nature, or that our isolated observations with *Vigna* establish that all of these proteins are active enzymes. We do believe, however, that the evidence now available demands that lectins be examined very carefully to determine if enzymically active forms might exist during stages of plant development other than the dormant seed stage.

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